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# Biodegradation and biotransformation of polycyclic non-steroidal anti-inflammatory drugs

Dorota Domaradzka · Urszula Guzik ·  
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**Abstract** In recent years the increased use of polycyclic non-steroidal anti-inflammatory drugs has resulted in their presence in the environment. This in turn may cause potential negative effects on living organisms. While the biotransformation mechanisms of polycyclic non-steroidal anti-inflammatory drugs in the human body and in other mammals have been extensively studied, degradation of these drugs by microorganisms has seldom been investigated and is largely unknown. Biotransformation/biodegradation of polycyclic non-steroidal anti-inflammatory drugs is caused by fungal microorganisms, mainly white-rot fungi, and a few strains of bacteria. However, hitherto only complete degradation of olsazine was described. The first step of the transformation is most often hydroxylation catalyzed by cytochrom P-450 monooxygenases, or oxygenation by laccases and three peroxidases: lignin peroxidase, manganese-dependent peroxidase and versatile peroxidase manganese-dependent peroxidase. The aim of this work is to summarize the knowledge about the biotransformation and/or biodegradation of polycyclic non-steroidal anti-inflammatory drugs and to present their biotransformation pathways.

**Keywords** Biodegradation · Biotransformation · Non-steroidal anti-inflammatory drugs · Microorganisms

## 1 Introduction

In recent years, the presence of polycyclic non-steroidal anti-inflammatory drugs (PNSAIDs) in the environment has been detected. The most popular of these drugs are naproxen (NPX), diclofenac (DCF), ketoprofen (KEP), mefenamic acid (MFA), flurbiprofen, indomethacin (IDM) and olsalazine (ADS), which are polycyclic nonsteroidal anti-inflammatory agents. These drugs have been detected in surface water, ground water and drinking water due to their extensive use in human and veterinary medicine (Razo-Flores et al. 1997; Ternes 1998; Heberer 2002, Heberer et al. 2002; Bright et al. 2011). Although these pharmaceuticals occur at concentrations within the  $\text{ng l}^{-1}$  to  $\mu\text{g l}^{-1}$  range and do not cause acute toxicity, they have a significant potential for biological activity such as chronic toxicity (Ternes 1998; Schwaiger et al. 2004; Quinn et al. 2008). Moreover, the removal of many pharmaceuticals in wastewater treatment plants has been found to be incomplete. That is why during the past few years much interest has arisen in the biotransformation of these contaminants (Thomas and Foster 2005; Gros et al. 2007). This review summarizes what is known about the biotransformation and/or biodegradation of polycyclic

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NSAIDs, and presents the transformation pathways of these pharmaceuticals.

Microbial biotransformation of polycyclic non-steroidal drugs is mainly caused by a few groups of microorganisms belonging to white-rot fungi (WRF). They are organisms which have the capability to degrade a wide range of pollutants, including polycyclic NSAIDs. The elimination of these pharmaceuticals by fungi can be carried out in two phases: surface binding on the fungal cell (adsorption), followed by a metabolism-dependent phase where compounds are transported into the cell and/or they are biotransformed outside the cell by extracellular enzymes. Extracellular enzymes are secreted basically as essential for lignin degradation. These nonspecific enzymes consist of laccases and three peroxidases: lignin peroxidase (LiP E.C. 1.11.1.14), manganese-dependent peroxidase (MnP, E.C.1.11.1.13) and versatile peroxidase (VP, E.C. 1.11.1.16). An advantage of the extracellular ligninolytic enzymes is the catalyzing of pharmaceuticals, even when the organisms are metabolically inactive. Moreover, WRF synthesize intracellular cytochrome P-450 monooxygenase, which intensifies their potential for degradation. Biotransformation of pharmaceuticals by WRF using the cytochrome P-450 system is similar to biotransformation by higher animals (Pointing 2001; Marco-Urrea et al. 2010a, b, c; Rodriguez-Rodriguez et al. 2013). PNSAID oxidation by this enzyme system leads to a new intermediate, mainly with the hydroxyl group. Apart from white-rot fungi the microbial transformation of polycyclic NSAIDs is also conducted by *Cunninghamella* species. These fungi possess cytochrome P-450 monooxygenase and enzymes analogous to mammalian enzymes of phase II which are responsible for drug conjugation with glucuronic acid or sulfuric acid (Asha and Vidyavathi 2009). Currently, there is a lack of information about the bacterial transformation/biodegradation of polycyclic non-steroidal anti-inflammatory drugs.

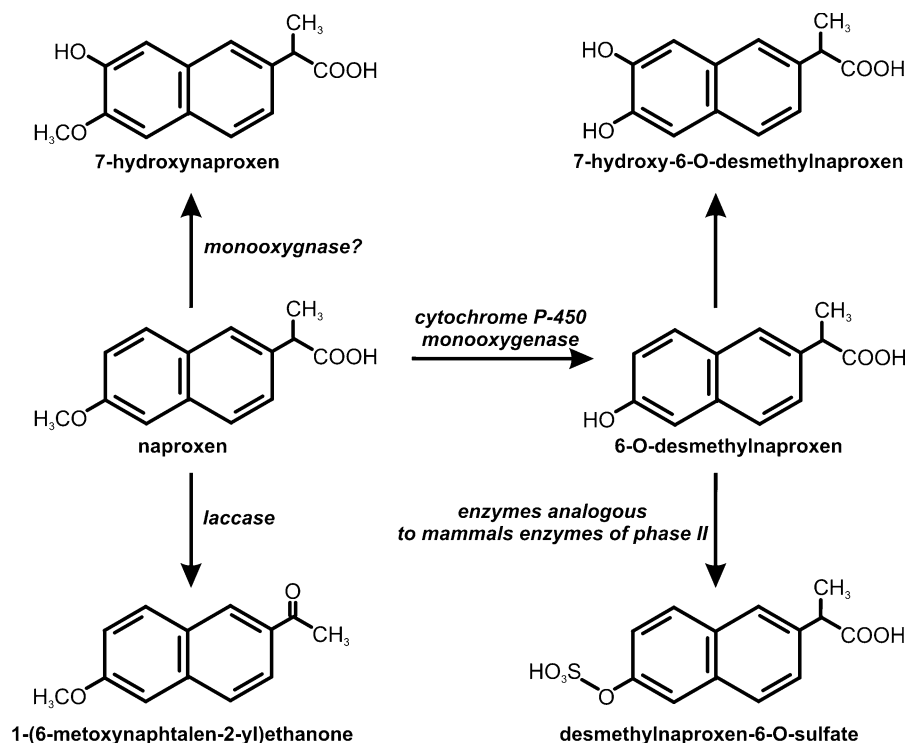
## 2 Naproxen biodegradation/biotransformation by microorganisms

Naproxen (2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid is one of the most widely used medicines for mild-to-moderate pain relief. Its presence in different environments is becoming a serious problem

because naproxen and its derivatives have adverse effects on biota, such as impairing the lipid peroxidation system of bivalves (Zheng et al. 2012). Marco-Urrea et al. (2010a) carried out almost complete biotransformation of naproxen by *Trametes versicolor* in 24 h. This drug was degraded at a concentration of  $10 \text{ mg l}^{-1}$  and  $55 \text{ } \mu\text{g l}^{-1}$ . A lower concentration was used because similar amounts of the pharmaceuticals are found in the environment. However, these authors observed 23.7 % naproxen adsorption on the surface of the fungi cells. The drug was catabolized by ligninolytic enzymes (laccases) as well as cytochrome P-450 in experimental flasks. Marco-Urrea et al. (2010a) identified the transformation products of naproxen: 6-*O*-desmethylnaproxen (2-(6-hydroxynaphthalen-2-yl)propanoic acid) and 1-(6-methoxynaphthalen-2-yl) ethanone. The first intermediate is created as a result of hydroxylation by cytochrome P-450 monooxygenase. After usage purified laccase solution in the second metabolite was observed (Marco-Urrea et al. 2010a) (Fig. 1).

It is known that polycyclic NSAIDs can be removed from wastewater treatment plants. However, these compounds are observed in influent and effluent. Moreover, polycyclic NSAIDs can also be adsorbed in the sewage sludge. This is why Rodriguez-Rodriguez et al. (2010a, b) investigated the degradation of naproxen in sewage sludge in wastewater treatment plants. Naproxen (to give a final concentration of  $\sim 0.096 \text{ mg g}^{-1}$  dry weight) was transformed by *T. versicolor* in sewage sludge in three different experiments. The first one consisted in the degradation of this pharmaceutical in a bottle containing a defined medium, autoclaved dry sewage sludge and glucose, which was periodically added (degradation in bioslurry systems). The second, was carried out in a flask containing dry sewage sludge, and wheat straw pellets as a lignocellulosic bulking material (degradation in solid-phase cultures). The third was composed of sewage sludge of different ages (10, 17, 25, 31, 38, and 45 days). In all experiments dry sewage sludge was sterilized before mycelium addition. The investigation of degradation in bioslurry systems has shown that the drug achieved maximum removal of around 47 %. Moreover, sorption–desorption equilibrium, which restores the naproxen concentration in the liquid phase, was observed. Naproxen transformation reached 56 % in the other experiments. *T. versicolor* in 17-day-old sewage sludge was the most efficient at

**Fig. 1** Microbiological transformation of naproxen (He and Rosazza 2003; Zhong et al. 2003; Marco-Urrea et al. 2010a; Rodarte-Morales et al. 2012)



transformation (Rodriguez-Rodriguez et al. 2010a, b): in younger and older than 17-day-old sludge biotransformation occurred to a lesser degree. Laccase activity showed a maximum in 17-day-old sewage sludge, but the activity of laccase was reduced during the cultivation period (Rodriguez-Rodriguez et al. 2010a, b). Tran et al. (2010) demonstrated that commercial laccase contributes to more efficient transformation of naproxen (100 %) than crude laccase isolated from *T. versicolor*. Also, Lloret et al. (2010) have shown that 60 % of naproxen is removed within 8 h by commercial laccase (2000 U l<sup>-1</sup>) from *Myceliophthora thermophila* with synthetic and natural mediators (Lloret et al. 2010; Tran et al. 2010).

Another white-rot fungus able to degrade pharmaceuticals is *Phanerochaete chrysosporium*. It causes the biotransformation of naproxen at an initial concentration of 2 mg l<sup>-1</sup>. After 30 days, this drug was almost totally removed (up to 97 %). *P. chrysosporium* has been grown in air and oxygen reactors with stirring and the addition of glucose. Although during the biotransformation of naproxen the activity of manganese-dependent peroxidase was observed, Rodarte-

Morales et al. (2012) suggested the engagement of cytochrome P-450 in this process. During this study two unidentified intermediates were isolated (Rodarte-Morales et al. 2012). Moreover, naproxen was transformed during 4 days in mixtures of drugs (diclofenac, ibuprofen and carbamazepine) added in a concentration of 1 mg l<sup>-1</sup> (Rodarte-Morales et al. 2011).

The transformation of naproxen by *Cunninghamella* species due to its cytochrome P-450 monooxygenase systems is noteworthy. *C. blakesleeana* and *C. echinulata* transformed naproxen into two metabolites after 96 h: 6-*O*-desmethylnaproxen and desmethylnaproxen-6-*O*-sulfate. Whereas *C. elegans* transformed naproxen only into 6-*O*-desmethylnaproxen. The authors suggested a part of the metabolic pathway of naproxen by *Cunninghamella* species, based on indicated metabolites and knowledge about mammal metabolism of naproxen (Fig. 1) (Zhong et al. 2003). He and Rosazza (2003) detected 6-*O*-desmethylnaproxen during the biotransformation of the drug by *Aspergillus niger*. Apart from that the authors indicated two previously unknown naproxen metabolites (He and Rosazza 2003): 7-hydroxynaproxen and 7-hydroxy-6-*O*-desmethylnaproxen (Fig. 1). 6-*O*-desmethylnaproxen was also

detected in a cometabolic culture of naproxen ( $5 \text{ mg l}^{-1}$ ) with powdered milk (Quintana et al. 2005) (Fig. 1).

Borras et al. (2011) carried out soil colonization with *T. versicolor* and a biotransformation experiment of naproxen over 24 h. They added lignocellulosis substrates such as: maize stalks, wheat straw, rice husks, pine sawdust, wheat straw pellets and two kinds of rabbit feedstock. Soil colonization was successful in sterile and non-sterile soil, while the presence of wheat straw pellets and two kinds of rabbit feedstock allowed higher laccase production. The biotransformation capacity of the fungus was higher when wheat straw pellets were employed, amounting to  $\sim 50 \%$ . A similar result was obtained during the colonization of sewage sludge in an investigation by Rodriguez-Rodriguez et al. (2010b). The success of the degradation process depends on both colonization of the soil and the degrading ability of the fungus throughout the treatment period.

### 3 Microbial degradation/transformation of diclofenac

Diclofenac (2-{2-[(2,6-dichlorophenyl)amino]phenyl}acetic acid) is a commonly used analgesic, anti-arthritic and anti-rheumatic NSAID, whose global consumption amounts to 940 tonnes per year (Coelho et al. 2009; Al-Rajab et al. 2010). Diclofenac is characterized by high durability and toxicity in the environment, despite known examples of biotransformation for this pharmaceutical (Quintana et al. 2005). Marco-Urrea et al. (2010a) investigated the biotransformation of diclofenac by *Trametes versicolor*, and they observed complete removal (94 %) at a concentration of  $10 \text{ mg l}^{-1}$  and  $45 \mu\text{g l}^{-1}$  (environmental concentration), while adsorption on the surface of the fungus cells of diclofenac was valued at 47 %. Diclofenac was biotransformed to three intermediates during 24 h. The authors suggest that the cytochrome P-450 system plays a key role in the transformation of diclofenac to 4'-hydroxydiclofenac and 5-hydroxydiclofenac. After the usage of purified laccase, these authors found 4-(2,6-dichlorophenylamino)-1,3-benzenedimethanol as a metabolite of diclofenac transformation (Fig. 2) (Marco-Urrea et al. 2010c). Also, *T. versicolor* was used to remove diclofenac from sewage

sludge with 64 % efficiency (Rodriguez-Rodriguez et al. 2011).

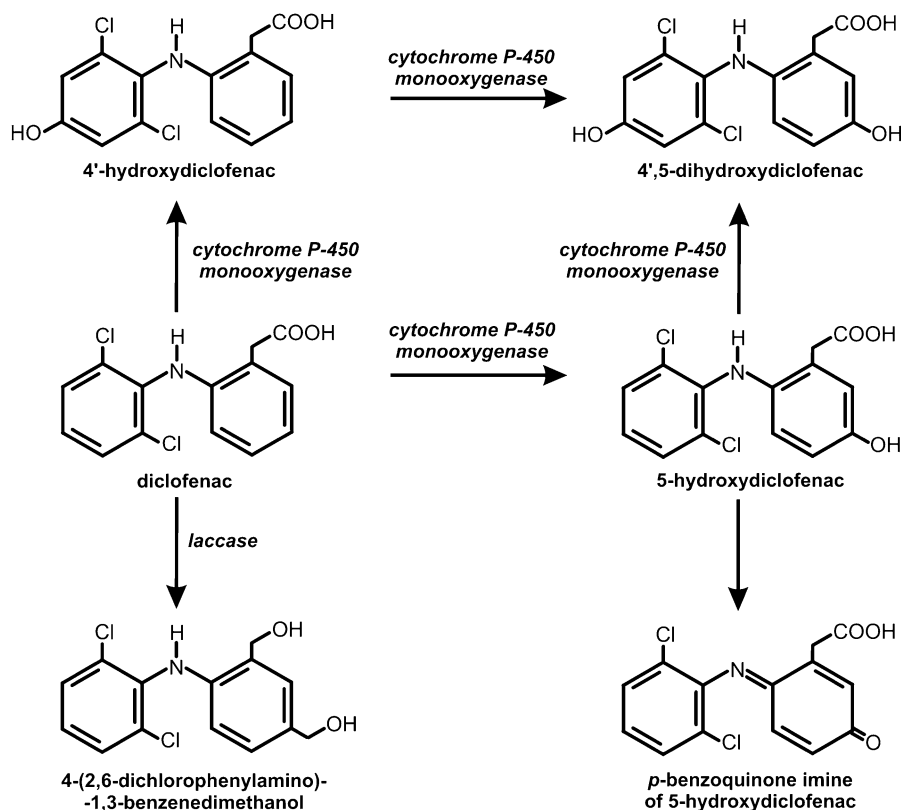
Degradation of polycyclic NSAIDs was also observed by *Phanerochaete chrysosporium* and *P. sordida* YK-624. *P. chrysosporium* transformed 93 % of diclofenac (initial concentration  $2 \text{ mg l}^{-1}$ ) after 30 days. Probably, manganese-dependent peroxidase was engaged in the biotransformation of these pharmaceuticals. However, the authors did not exclude the role of cytochrome P-450 in this process. During this study, two intermediates were isolated but they were not identified. In the culture of the second strain—*P. sordida* YK-624 with diclofenac 4'-hydroxydiclofenac (predominant metabolite), 5-hydroxydiclofenac and 4',5-dihydroxydiclofenac were identified. Metabolites formed via fungal transformation suggested that hydroxylation was catalyzed by monooxygenases cooperating with cytochrome P-450. However, the authors did not exclude the role of extracellular ligninolytic enzymes in diclofenac transformation (90 % during 3 days) (Hata et al. 2010; Rodarte-Morales et al. 2012). The same metabolites were indicated in a culture of bacteria—*Actinoplanes* sp. Osorio-Lozada et al. (2008) investigated the transformation of diclofenac by seven strains of bacteria and 8 strains of fungi. Only two bacterial strains and five fungal strains were able to biotransform of drug. *Actinoplanes* sp. hydroxylated diclofenac ( $50 \mu\text{M}$ ) more efficiently than other strains (100 % of diclofenac during 5 h), whereas *Cunninghamella elegans*, *Cunninghamella echinulata* and *Beauveria bassiana* transformed 100 % of this drug within 120 h (Osorio-Lozada et al. 2008).

4'-Hydroxydiclofenac also appeared in a large amount from biotransformation by recombinant *Escherichia coli* and *Epiccocum nigrum* IMI354292. *Pestalotiopsis* sp. IMI353656 and *E. nigrum* IMI356573 produced small amounts of 3'-hydroxydiclofenac and 5-hydroxydiclofenac, along with 4'-hydroxydiclofenac, while *Pestalotiopsis* sp. IMI35348 transformed diclofenac to 5-hydroxydiclofenac and 4'-hydroxydiclofenac (Webster et al. 1998; Vail et al. 2005).

Groning et al. (2007) investigated diclofenac degradation in river sediment. The authors assayed intermediates of diclofenac: 4'-hydroxydiclofenac, 5-hydroxydiclofenac and the major metabolite of 5-hydroxydiclofenac—*p*-benzoquinone imine. Enzymes were not indicated, but it



**Fig. 2** Transformation of diclofenac by microorganisms (Groning et al. 2007; Marco-Urrea et al. 2010c)



was suggested that *p*-benzoquinone imine was formed out of 5-hydroxydiclofenac by dehydration (Fig. 2) (Groning et al. 2007).

Removal of diclofenac by microorganisms from a mixture of selected pharmaceuticals was also reported. 99 % of this drug (concentration of the drug mixture:  $\sim 1 \text{ mg l}^{-1}$ ) was transformed within 4 days by *Bjerkandera* sp. R1, while *P. chrysosporum* and *Bjerkandera adusta* transformed 12 and 9 % of diclofenac within 7 days, respectively. However, the authors did not observe and did not identify metabolites (Rodarte-Morales et al. 2011).

Diclofenac transformation was also observed by pure enzymes—laccase—from cell free extract of *T. versicolor*, and commercial laccase from *T. versicolor*. It was observed that the degradation of diclofenac increased with the increase of laccase activity, or through the addition of redox mediators such as syringaldehyde. Moreover, it was suggested that the presence of nitrogen and the negative charge in the diclofenac ring may facilitate the degradation of these compounds by laccase (Lloret et al. 2010; Tran et al. 2010).

#### 4 Biodegradation/biotransformation of ketoprofen

Ketoprofen 2-(3-benzoylphenyl)propanoic acid is a type of NSAID which has been detected in soil, surface water and in influents and effluents of wastewater treatment plants in concentrations ranging from  $\text{ng l}^{-1}$  up to  $\mu\text{g l}^{-1}$  (Xu et al. 2009; Marco-Urrea et al. 2010b; Almeida et al. 2013). Adsorption and transformation of ketoprofen in soil are among the processes dependent on biotic and abiotic factors in soil. Xu et al. (2009) showed that ketoprofen was not strongly adsorbed in soil. These authors showed that degradation of the drug depends on physical and chemical properties of soil, total soil microbial activity, and bioavailability, as well as inheritance of light intensity (Matamoros et al. 2009; Xu et al. 2009).

It has been shown that ketoprofen may be transformed by microorganisms, mainly by fungi. Marco-Urrea et al. (2010b) showed that *Trametes versicolor* biotransformed ketoprofen at a concentration of  $40 \mu\text{g l}^{-1}$  (the concentration of ketoprofen in the environment) and  $10 \text{ mg l}^{-1}$ . They observed 15 %

adsorption of ketoprofen on the surface of fungal cells. Probably, ketoprofen degradation occurred with the usage of cytochrome P-450. Extracellular activities of laccase were detected, but transformation of the drug was not observed in a study with purified laccase. During the transformation of ketoprofen by whole fungal cells, crude laccase and commercial laccase, Tran et al. (2010) observed 100, 25, and 50 % transformation, respectively. This suggests that the biotransformation of the drug by whole fungal cultures may probably also be effected by intracellular oxidizing activities (Tran et al. 2010).

Marco-Urrea et al. (2010b) identified three metabolites of ketoprofen transformation by *Trametes versicolor*: 2-[(3-hydroxy(phenyl)methyl)phenyl]-propanoic acid, the major degradation metabolite formed by reduction of the ketone group, and 2-(3-benzoyl-4-hydroxyphenyl)propanoic acid and/or 2-(3-(4-hydroxybenzoyl)phenyl)propanoic acid, which were formed by hydroxylation (Fig. 3).

Quintana et al. (2005) proposed the degradation pathway of ketoprofen by activated sludge. Based on these metabolites, the authors proposed that ketoprofen is degraded along the pathway known for biphenyls, biphenyl ethers and related compounds. The first step of ketoprofen degradation was the reduction of the keto group. This led to electron density of the ring, which was more reactive towards electrophilic dioxygenation. As a consequence, the respective catechol derivatives were formed, which undergo extradiol cleavage to hydroxymuconic semialdehyde. This intermediate was hydrolysed to hydroxyl-pentadienoic acid and 3-(hydroxyl-carboxymethyl) hydratopic acid. These compounds oxidized to 3-(keto-carboxymethyl) hydratopic acid (Fig. 3) (Quintana et al. 2005).

## 5 Mefenamic acid, indomethacin and flurbiprofen biodegradation/biotransformation by microorganisms

Mefenamic acid (2-[(2,3-dimethylphenyl)amino]benzoic acid), indomethacin ((2-{1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl}acetic acid)) and flurbiprofen ((2-(3-fluoro-4-biphenyl)propanoic acid)) are used less often than the earlier described NSAIDs. However, they and their metabolites are detected in the aquatic environment (Chowdhury et al. 2003; Amadio et al. 2010; Hata et al. 2010).

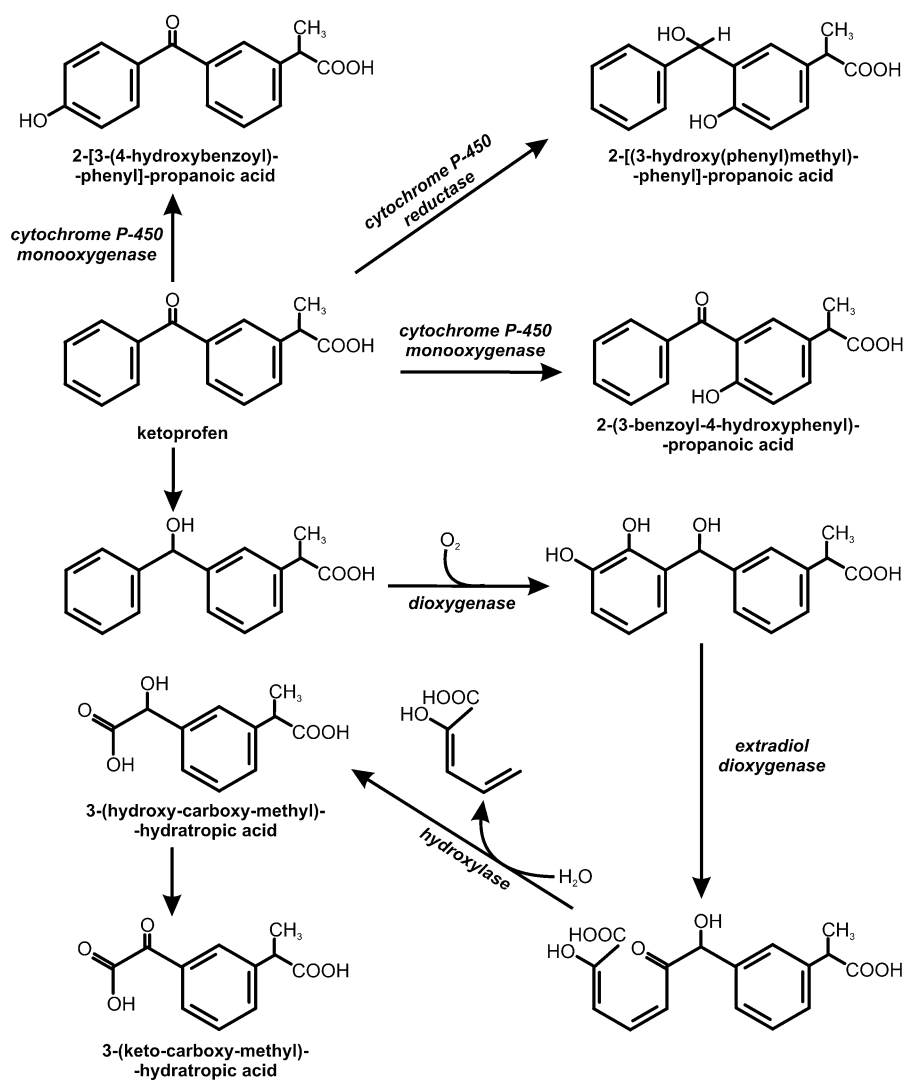
Mefenamic acid exhibits a high ability of binding to solids (Jones et al. 2006). Nevertheless, it was up to 72 % removed by sewage sludge with *T. versicolor*, and a strong decrease in toxicity was observed. During this study laccase activity was indicated, but its level was variable (Rodriguez-Rodriguez et al. 2011).

Hata et al. (2010) examined the biotransformation of mefenamic acid by *Phanerochaete sordida* YK-624. During this study, they observed about 60 and 90 % depletion of the introduced dose after 3 and 6 days of treatment, respectively. The appearance of four intermediates: 3'-hydroxymefenamic acid, 3'-carboxymefenamic acid, 3'-hydroxymethyl-6'-hydroxymefenamic acid and 3'-hydroxymethyl-5'-hydroxymefenamic acid (Fig. 4) suggests that the cytochrome P-450 system is responsible mainly for the elimination of mefenamic acid. However, the roles of laccase, manganese-dependent peroxidase and lignin peroxidase in the transformation of ketoprofen could not be excluded (Fig. 4) (Hata et al. 2010).

It is known that flurbiprofen shows a strong antifungal activity (Chowdhury et al. 2003). In the environment, fungi which able to transform this drug are nevertheless found. Amadio et al. (2010) revealed flurbiprofen transformation to 4'-hydroxyflurbiprofen and 3'-hydroxy-4'-methoxyflurbiprofen, as well as to 3',4'-dihydroxyflurbiprofen by *Cunninghamella* species: *C. elegans* DSM 1908, *C. echinulata* DSM 1905 and *C. blakesleeana* DSM 1906. *C. elegans* strains DSM 8217 and DSM 63299 were able to transform flurbiprofen only to 4'-hydroxyflurbiprofen and 3'-hydroxy-4'-methoxyflurbiprofen. The appearance of these metabolites allows us to speculate on the hydroxylating role of the cytochrome P-450 systems in the biotransformation of flurbiprofen. Moreover, *C. elegans* DSM 1908 and *C. blakesleeana* DSM 1906 also produced a sulfated drug—an analog of the II phase metabolite of the mammalian detoxification system (Fig. 5) (Amadio et al. 2010).

Also bacteria take part in the biological transformation of flurbiprofen. Bright et al. (2011) conducted its transformation in cultures of *Streptomyces* sp. The intermediates 4'-hydroxyflurbiprofen, 3',4'-dihydroxyflurbiprofen and 3'-methoxy-4'-hydroxyflurbiprofen were detected in the culture medium of *S. griseus* ATCC 13273 and *S. griseus* DSM 40236. *S. griseus* DSM 40226 and *S. rimosus* DSM 40260 hydroxylated flurbiprofen to 4'-hydroxyflurbiprofen, while *S. subtritis* DSM 40445 transformed the examined compound

**Fig. 3** Biotransformation of ketoprofen (Quintana et al. 2005; Marco-Urrea et al. 2010b)



to 4'-hydroxyflurbiprofen and 3'-methoxy-4'-hydroxyflurbiprofen. The enzymes engaged in flurbiprofen transformation are probably analogs of mammalian cytochromes P-450. Moreover, *S. lavenduligriseus* DSM 40487, *Bacillus subtilis* IM7, *B. megaterium* NCIMB8291 and *B. megaterium* ATCC14581 produced flurbiprofenamide and 7-hydroxyflurbiprofenamide as metabolites of flurbiprofen (Fig. 5) (Bright et al. 2011).

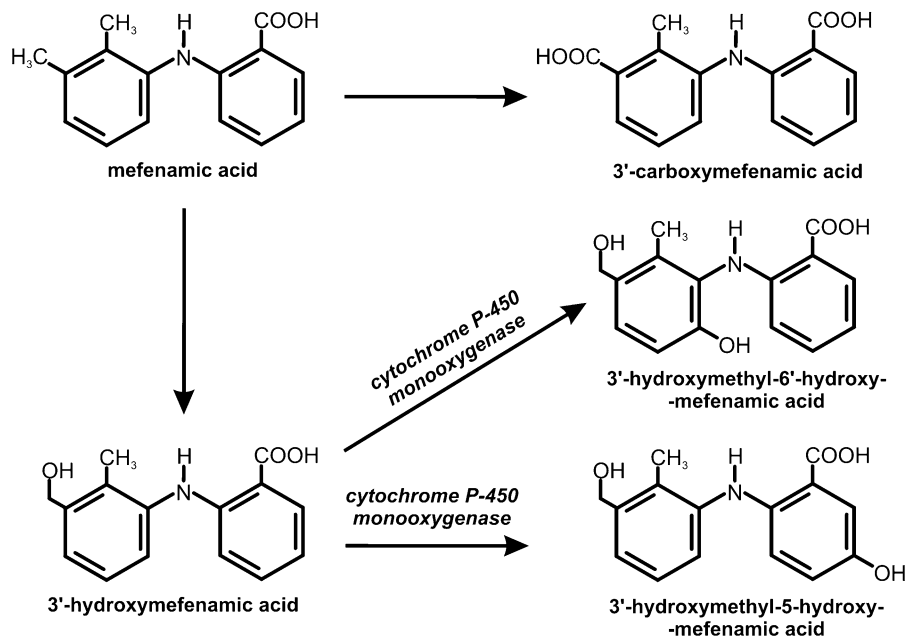
Zhang et al. (2006) examined the biotransformation of indomethacin by *Cunninghamella blakesleeana* AS 3.910. The drug was partially metabolized (87.41 %) to three metabolites: *O*-desmethyindomethacin, *N*-deschlorobenzoylindomethacin and *O*-desmethyl-*N*-deschlorobenzoylindomethacin. Transformation of

the drug was also carried out by *C. elegans* AS 3.156 (38.76 %), *C. elegans* AS 3.2028 (69.29 %), *C. blakesleeana* AS 3.153 (51.90 %) and *C. echinulata* AS 3.2004 (39.52 %), but metabolites of indomethacin were not identified (Zhang et al. 2006) (Fig. 6).

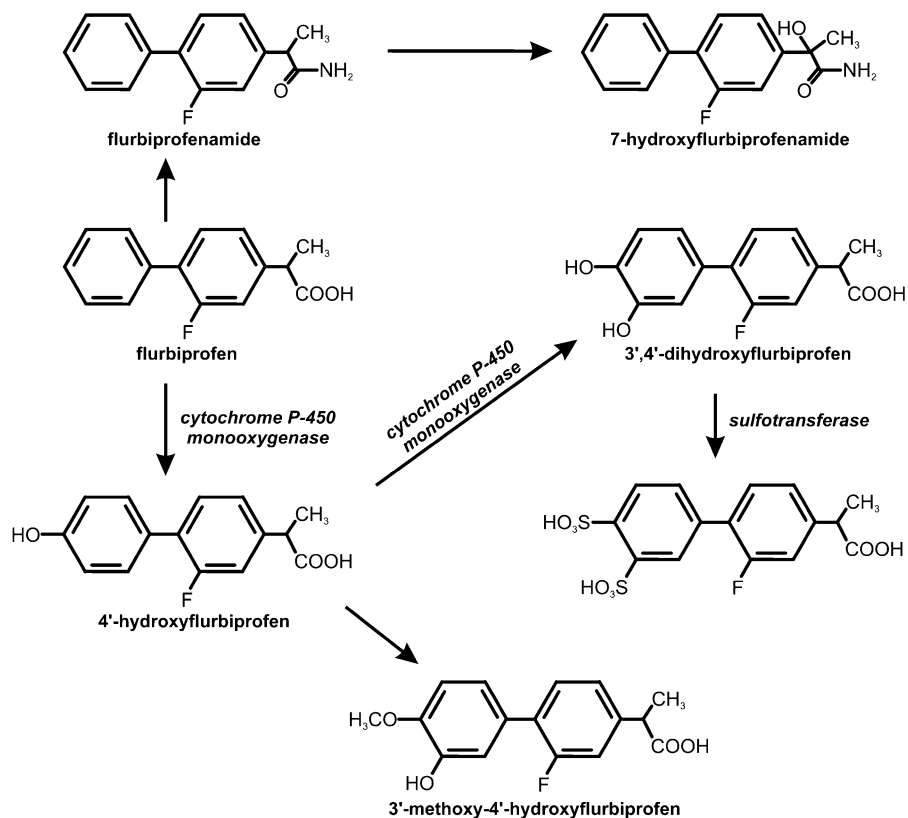
Tran et al. (2010) observed the complete biotransformation of indomethacin by a whole fungal culture of *T. versicolor*. The experiment with crude laccase and commercial laccase showed that this enzyme is probably engaged in the biotransformation process. The nitrogen atom in the structure of indomethacin accelerates the reaction with laccase (Tran et al. 2010).



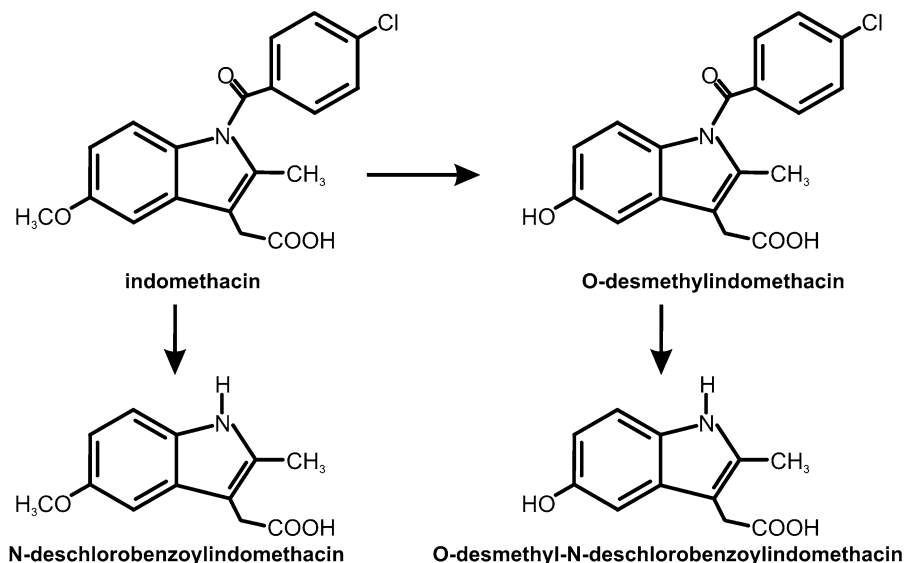
**Fig. 4** Biotransformation of mefenamic acid by *Phanerochaete sordida* YK-624 (Hata et al. 2010)



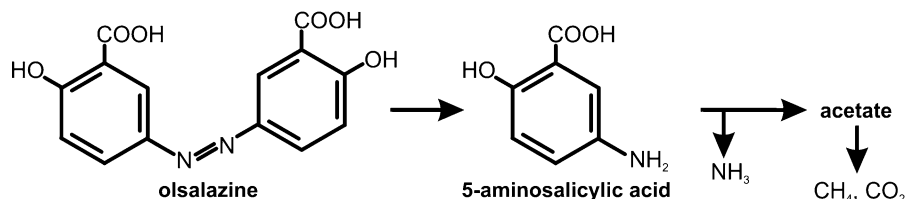
**Fig. 5** Microbiological transformation of flurbiprofen (Amadio et al. 2010; Bright et al. 2011)



**Fig. 6** Biotransformation of indomethacin by fungi (Zhang et al. 2006)



**Fig. 7** Biotransformation of olsalazine by methanogenic consortium (Razo-Flores et al. 1997)



## 6 Degradation of olsalazine as an example of NSAIDs from the azo dye group

Olsalazine (5-[(E)-2-(3-carboxy-4-hydroxyphenyl)diazen-1-yl]-2-hydroxybenzoic acid) is known as azodisalicylate and belongs to the azo dye group. This drug is resistant to aerobic transformations by bacteria because the azo group has a strong electron-withdrawing character and stabilizes this aromatic compound against conversion by oxygenases (Keck et al. 1997). Razo-Flores et al. (1997) described the 89 % transformation of olsalazine by a methanogenic consortium immobilized in sludge granules during 7 months under anaerobic conditions. Glucose and a mixture of acetate, propionate, and butyrate were used as the co-substrate to provide the electrons for the olsalazine reductive cleavage. During experiments, the authors assayed the metabolite, 5-aminosalicylic acid, which was created throughout azo bond reduction. In the subsequent biotransformation period, 5-aminosalicylic acid was extensively mineralized by the methanogenic consortium to methane and

ammonium, which were observed in the bioreactors. When 5-aminosalicylic acid was incubated with a specific methanogenic inhibitor, acetate was identified as the major intermediate of olsalazine (Fig. 7) (Razo-Flores et al. 1997).

## 7 Conclusion

The polycyclic non-steroidal anti-inflammatory drugs present in wastewater can be biotransformed by microorganisms, but only olsalazine is completely degraded. White-rot fungi are a group of microorganism with a large ability to transformation polycyclic NSAIDs. *T. versicolor* could be used as an agent for the degradation of mainly pharmaceuticals, but it still requires further studies. The presented pathways of biotransformation for selected drugs are not complete. In many case enzymes were not indicated, or it is only speculation about their activity. There are known physical–chemical treatment methods which usually achieve high removal efficiencies. However, they

sometimes lead to the accumulation of intermediates more toxic than the parent compounds. Biological degradation could be more economically and environment friendly, but it requires knowledge of the polycyclic NSAIDs degradation pathways, and not just the biotransformation pathways.

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